APPENDIX A

NICEATM-DEFINED RETROSPECTIVE *IN VIVO* OCULAR IRRITANCY CLASSIFICATION RULES

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NICEATM-Defined Retrospective *In Vivo* Ocular Irritancy Classification Rules

Regulatory Agency	Number of Rabbits	Observation Times (after treatment)	Mean Score Used?	Basis for a Positive Response	Classification Categories
EPA	Tests could be based on a single rabbit (if marked effects were expected), otherwise 3 to 6 rabbits	1 hour, and 1, 2, 3, 7, 14, and 21 days; if no irritation after 3 days study may be ended	No	Opacity or Iritis ≥ 1 or Redness or Chemosis ≥ 2	At least 1 positive rabbit needed for classification: **I** = Corrosive, corneal involvement, or irritation persisting more than 21 days **II**= Corneal involvement or irritation clearing* in 8-21 days **III** = Corneal involvement or irritation clearing* in 7 days or less **IV** = Minimal effects clearing* in less than 24 hours *Clearing: Opacity and Iritis = 0; Redness and Chemosis is 0 or 1
European Union	Single rabbit if marked effects expected; Sequential testing of rabbits until response is confirmed (typically, up to 3 rabbits)	Minimum observation times of 1, 2, and 3 days; Additional observation times may be performed at 7, 14, and 21; If no irritation after 3 days study may be ended.	Yes	**More than 3 rabbits:** Averaged score of all animals tested for each endpoint over Days 1, 2, and 3 **Three rabbits:** Individual rabbit endpoint scores averaged over Days 1, 2, and 3	**Classification is sequential (i.e., first determine if meets R41 criteria, then R36 criteria, any substance remaining is classified as nonirritant if test adequate)** **R41 Classification (follow rules sequentially)** (1) When more than 3 rabbits tested, where mean study value for Opacity ≥ 3 and/or Iritis > 1.5 (2) When 3 rabbits tested, when 2 rabbits have individual animal mean values where Opacity ≥ 3 and/or Iritis = 2 ***Regardless of the number of rabbits tested*** (3) At least 1 of 3 (or 2 of 6) rabbits on Day 21 where effects have not reversed to 0 (4) If study ends on or after Day 14 and before Day 21, at least 1 of 3 (2 of 4, 5, 6) rabbits where Opacity ≥ 3 and/or Iritis = 2 (5) At least one rabbit where study is terminated early and there is (a) corneal perforation or significant corneal ulceration including staphyloma, (b) blood in the anterior chamber of the eye, (c) Opacity = 4 which persists for 48 hours, (d) absence of light reflex (Iritis = 2) which persists for 72 hours, (e) ulceration of the conjunctival membrane, (f) necrosis of the conjunctivae or nictitating membrane, or (g) sloughing. (Notes should be clear that early termination was due to one of these effects) **R36 Classification (follow rules sequentially)** (1) When more than 3 rabbits tested, where mean study values are: ≥ 2 Opacity < 3, ≥ 1 Iritis < 1.5, Redness ≥ 2.5, and/or Chemosis ≥ 2 (2) When 3 rabbits tested, if 2 rabbits have individual animal mean values where ≥ 2 Opacity < 3, ≥ 1 Iritis < 2, Redness ≥ 2.5, and/or Chemosis ≥ 2

Regulatory Agency	Number of Rabbits	Observation Times (after treatment)	Mean Score Used?	Basis for a Positive Response	Classification Categories
					Nonirritant (1) When a substance cannot be classified as R41 or R36 and the test is adequate
GHS	Sequential testing of rabbits until response is confirmed (typically, up to 3 rabbits)	1, 2, 3 days (if effects induced, observation until reversal or Day 21, whichever comes first)	Yes	Individual rabbit values averaged over Days 1, 2, and 3	**Classification is sequential (i.e., first determine if meets Category 1 criteria, then Category 2 criteria, any substance remaining is classified as nonirritant if test adequate)** **Category 1 (Irreversible Eye Effects) (follow rules sequentially)** (1) At least 2 of 3 (or 4 of 6; 3 of 4; 4 of 5) rabbits have individual animal mean values where Opacity ≥ 3 and/or Iritis > 1.5 (2) At least 1 of 3 (2 of 4, 5, 6) rabbits where Opacity, Chemosis, Redness, and/or Iritis > 0 on Day 21 (3) 1 of 6 rabbits has an animal mean value where Opacity ≥ 3 and/or Iritis > 1.5 and 1 of 6 rabbits (a different rabbit) where Opacity, Chemosis, Redness, and/or Iritis > 0 on Day 21 (4) At least 1 of 3 (2 of 4, 5, 6) rabbits with an Opacity = 4 at any time (5) If study ends on or after Day 14, at least 1 rabbit where Opacity = 3 and/or Iritis = 2 (6) At least 1 rabbit where study is terminated early and there is (a) corneal perforation or significant corneal ulceration including staphyloma, (b) blood in the anterior chamber of the eye, (c) absence of light reflex (Iritis =2) which persists for 72 hours, (d) ulceration of the conjunctival membrane, (e) necrosis of the conjunctivae or nictitating membrane, or (f) sloughing. (Notes should be clear that early termination was due to one of these effects) **Category 2 (Reversible Eye Effects) (follow rules sequentially)** (1) Eye Irritant Category 2A - At least 2 of 3 (or 4 of 6; 3 of 4; 4 of 5) rabbits have individual animal mean values where ≥ 1 Opacity <3, ≥ 1 Iritis ≥ 1.5, Redness ≥ 2, and/or Chemosis ≥ 2 and the effect reverses > 7 days but < 21 days. (2) Eye Irritant Category 2B - At least 2 of 3 (or 4 of 6; 3 of 4; 4 of 5) rabbits have individual animal mean values where ≥ 1 Opacity <3, ≥ 1 Iritis ≥ 1.5, Redness ≥ 2, and/or Chemosis ≥ 2 and the effect reverses by 7 days **Nonirritant (follow rules sequentially)** (1) When a substance cannot be classified as Category 1 or 2 and the test is adequate

Regulatory Agency	Number of Rabbits	Observation Times (after treatment)	Mean Score Used?	Basis for a Positive Response	Classification Categories
					Study Criteria Not Met (1) When 1 of 6 animals falls into Category 1 rule (1) or (4) and other 5 animals are nonirritant (2) When no more than 3 animals fall into Category 1 rule (2) and other animals are nonirritant or Category 2

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